

WHAT IS CLAIMED IS:

1 1. A ligand that binds specifically to a region of a polymeric
2 immunoglobulin receptor (pIgR) of a cell of an animal, which pIgR when cleaved has a
3 stalk region which remains attached to the cell and a secretory component (SC) which
4 exists in an organ of interest in several forms, provided that the ligand does not
5 substantially bind to the most abundant form of SC present in the organ of interest and
6 provided further that the ligand does not substantially bind to the stalk of said pIgR under
7 physiological conditions.

1 2. A ligand of claim 1 in which the animal is a bird.

1 3. A ligand of claim 1 in which the animal is a mammal.

1 4 A ligand of claim 3 in which the mammal is selected from the
2 group consisting of pig, cow, horse, sheep, goat, cat, dog, and human.

1 5. A ligand of claim 1 wherein the ligand is an antibody.

1 6. A ligand of claim 1 wherein the ligand is a humanized antibody.

1 7. A ligand of claim 1 wherein the ligand is selected from the group
2 consisting of a recombinant single chain variable region fragment of an antibody and a
3 disulfide stabilized variable region fragment.

1 8. A ligand of claim 1 which binds to a peptide derived from human
2 pIgR (SEQ ID NO:2), which peptide is selected from the group consisting of: Lys487-
3 Arg603, Lys487-Glu607, Lys487-Val611, Lys487-Arg615, Lys487-Ala618, Cys520-
4 Arg603, Cys520-Glu607, Cys520-Val611, Cys520-Arg615, Cys520-Ala618, Lys577-
5 Arg603, Lys577-Glu607, Lys577-Val611, Lys577-Arg615, Lys577-Ala618, Ser574-
6 Arg603, Ser574-Glu607, Ser574-Val611, Ser574-Arg615, Ser574-Ala618, Val560-
7 Arg603, Val560-Glu607, Val560-Val611, Val560-Arg615, Val560-Ala618, Cys544-
8 Arg603, Cys544-Glu607, Cys544-Val611, Cys544-Arg615, and Cys544-Ala618.

1 9. The ligand of claim 1, further wherein the ligand binds to an
2 epitope selected from the group consisting of QDPRLF (SEQ ID NO:10), LDPRLF (SEQ
3 ID NO:11), KAIQDPRLF (SEQ ID NO:12), LDPRLFADERI (SEQ ID NO:13),

SUB
A16

4 DENKANLDPRLF (SEQ ID NO:14), RLFADEREI (SEQ ID NO:15), and LDPRLFADE
5 (SEQ ID NO:16).

1 10. The ligand of claim 1, wherein the organ of interest is selected
2 from the group consisting of a small intestine, a large intestine, a liver-biliary tree, a
3 salivary gland, a stomach, a lung, a vagina, a uterus, a lacrimal gland, a mammary gland,
4 a nasal passage, and a sinus.

1 11. A ligand of claim 1 which is further defined as comprising a
2 binding component for binding to pIgR and a biologically active component.

1 12. A ligand of claim 11, wherein the organ of interest is the lung.

1 13. A ligand of claim 12, wherein the biologically active component is
2 a nucleic acid encoding the wildtype cystic fibrosis transmembrane conductance
3 regulator.

1 14. A ligand of claim 11, wherein the biologically active component is
2 selected from the group consisting of a nucleic acid, a protein, a radioisotope, a lipid, a
3 carbohydrate, a peptidomimetic, an anti-inflammatory, an antibiotic, and an anti-infective.

1 15. A ligand of claim 11, wherein the biologically active component is
2 a small molecule.

1 16. A ligand that binds specifically to a region of a polymeric
2 immunoglobulin receptor (pIgR) of a cell of an animal, which pIgR has an initial
3 cleavage site and which upon initial cleavage has a stalk region which remains attached to
4 the cell and a secretory component (SC) which exists in an organ of interest in several
5 forms, provided that the ligand does not substantially bind to the most abundant form of
6 SC present in the organ of interest and provided further that the ligand does not
7 substantially bind to a peptide comprising 31 amino acids that are cell-membrane-
8 proximal to the initial cleavage site.

1 17. A method of introducing a ligand into a cell of an organ of interest
2 in an animal, which cell expresses a polymeric immunoglobulin receptor, by binding the
3 ligand to a region of the polymeric immunoglobulin receptor, with the provisos that
4 (a) the ligand does not substantially bind to a form of secretory component

5 which is the most abundant form present in the organ of interest under physiological
6 conditions and

7 (b) the ligand does not substantially bind to a stalk region of the pIgR,
8 thereby permitting introduction of the ligand into the cell.

1 18. A method of claim 17, wherein the ligand is an antibody.

1 19. A method of claim 17, wherein the ligand is a humanized antibody.

1 20. A method of claim 17, wherein the ligand is selected from the
2 group consisting of a recombinant single chain variable region fragment of an antibody
3 and a disulfide stabilized variable region fragment.

1 21. A method of claim 17, wherein the ligand selectively binds to a
2 peptide derived from human pIgR (SEQ ID NO:2), which peptide is selected from the
3 group consisting of: Lys487-Arg603, Lys487-Glu607, Lys487-Val611, Lys487-Arg615,
4 Lys487-Ala618, Cys520-Arg603, Cys520-Glu607, Cys520-Val611, Cys520-Arg615,
5 Cys520-Ala618, Lys577-Arg603, Lys577-Glu607, Lys577-Val611, Lys577-Arg615,
6 Lys577-Ala618, Ser574-Arg603, Ser574-Glu607, Ser574-Val611, Ser574-Arg615,
7 Ser574-Ala618, Val560-Arg603, Val560-Glu607, Val560-Val611, Val560-Arg615,
8 Val560-Ala618, Cys544-Arg603, Cys544-Glu607, Cys544-Val611, Cys544-Arg615, and
9 Cys544-Ala618.

1 22. The method of claim 17, wherein the ligand binds to an epitope
2 selected from the group consisting of QDPRLF (SEQ ID NO:10), LDPRLF (SEQ ID
3 NO:11), KAIQDPRLF (SEQ ID NO:12), LDPRLFADERI (SEQ ID NO:13),
4 DENKANLDPRLF (SEQ ID NO:14), RLFADEREI (SEQ ID NO:15), and LDPRLFAD
5 (SEQ ID NO:16).

1 23. A method of claim 17, wherein the ligand is further defined as
2 having a binding component for selectively binding to pIgR and a biologically active
3 component.

1 24. A method of claim 23, wherein the biologically active component
2 is a nucleic acid which encodes the wildtype cystic fibrosis transmembrane conductance
3 regulator.

1 25. A method of claim 17, wherein the biologically active component
2 is selected from the group consisting of a nucleic acid, a protein, a radioisotope, a lipid, a
3 carbohydrate, a peptidomimetic, an anti-inflammatory, an antibiotic, and an anti-infective.

1 26. A method of claim 17, wherein the biologically active component
2 is a small molecule.

1 27. A method of claim 17, wherein the cell is a mammalian cell.

1 28. A method of claim 27, wherein the cell is an epithelial cell.

1 29. A method of claim 17, wherein the organ of interest is selected
2 from the group consisting of a small intestine, a large intestine, a liver-biliary tree, a
3 stomach, a salivary gland, a lung, a vagina, a uterus, a lacrimal gland, a mammary gland,
4 a nasal passage, and a sinus.

1 30. A method of introducing a ligand into a cell of an organ of interest
2 in an animal, which cell expresses a polymeric immunoglobulin receptor (pIgR), which
3 pIgR has an initial cleavage site which, upon initial cleavage has a stalk region, the
4 method comprising binding the ligand to a region of the pIgR, with the provisos that

5 (a) the ligand does not substantially bind to a form of secretory component
6 which is the most abundant form present in the organ of interest under physiological
7 conditions;

8 (b) the ligand does not substantially bind to a stalk region of the pIgR; and

9 (c) the ligand does not bind to an extracellular epitope within the first 31
10 amino acids that are cell membrane proximal to the initial cleavage site of the pIgR,
11 thereby permitting introduction of the ligand into the cell.

1 31. A method of increasing the rate by which a first ligand which binds
2 to secretory component (SC) is internalized into a cell secreting a polymeric
3 immunoglobulin receptor (pIgR) from an apical surface by

4 (a) binding the pIgR with a second ligand, which second ligand inhibits
5 proteolytic cleavage of SC by at least one-third, and further which second ligand does not
6 substantially bind to a stalk remaining attached to the cell after proteolytic cleavage, and

7 (b) binding the first ligand to the SC,

8 thereby permitting internalization into said cell of the SC to which the first ligand is
9 bound.

1 32 A ligand that binds specifically to a region of a polymeric
2 immunoglobulin receptor (pIgR) of a cell, provided that binding of the ligand reduces
3 proteolytic cleavage of secretory component (SC) by at least one-third compared to the
4 cleavage of SC from a cell in the absence of binding of the ligand and provided further
5 that the ligand does not substantially bind to a stalk of said pIgR remaining after
6 proteolytic cleavage under physiological conditions.

1 33. A ligand of claim 32, wherein the ligand is an antibody.

1 34. A ligand of claim 33, wherein the ligand is a humanized antibody.

1 35. A ligand of claim 33, wherein the ligand is a scFv.

1 36. A ligand of claim 33, wherein the ligand is selected from the group
2 consisting of a recombinant single chain variable region fragment of an antibody and a
3 disulfide stabilized variable region fragment.

1 37. A ligand of claim 32, which binds to a peptide derived from human
2 pIgR (SEQ ID NO:2), selected from the group consisting of: Lys487-Arg603, Lys487-
3 Glu607, Lys487-Val611, Lys487-Arg615, Lys487-Ala618, Cys520-Arg603, Cys520-
4 Glu607, Cys520-Val611, Cys520-Arg615, Cys520-Ala618, Lys577-Arg603, Lys577-
5 Glu607, Lys577-Val611, Lys577-Arg615, Lys577-Ala618, Ser574-Arg603, Ser574-
6 Glu607, Ser574-Val611, Ser574-Arg615, Ser574-Ala618, Val560-Arg603, Val560-
7 Glu607, Val560-Val611, Val560-Arg615, Val560-Ala618, Cys544-Arg603, Cys544-
8 Glu607, Cys544-Val611, Cys544-Arg615, and Cys544-Ala618.

1 38. The ligand of claim 32, wherein the ligand binds to an epitope
2 selected from the group consisting of QDPRLF (SEQ ID NO:10), LDPRLF (SEQ ID
3 NO:11), KAIQDPRLF (SEQ ID NO:12), LDPRLFADERI (SEQ ID NO:13),
4 DENKANLDPRLF (SEQ ID NO:14), RLFADEREI (SEQ ID NO:15), and LDPRLFAD
5 (SEQ ID NO:16).

1 39 A ligand of claim 32 which is further defined as a binding
2 component of a molecule comprising a biologically active component.

1 40. A ligand of claim 39, wherein said biologically active component
2 is selected from the group consisting of: a nucleic acid, a protein, a radioisotope, a lipid, a
3 carbohydrate, a peptidomimetic, an anti-inflammatory, an antibiotic, and an anti-infective.

1 41. A ligand of claim 39, wherein the biologically active component is
2 a small molecule.

1 42. A ligand of claim 39, wherein the biologically active component is
2 a nucleic acid encoding the wildtype cystic fibrosis transmembrane conductance
3 regulator.

1 43. A conjugate, fusion protein, or complex, said conjugate fusion
2 protein or complex comprising a ligand that binds specifically to a region of a polymeric
3 immunoglobulin receptor (pIgR) of a cell and a biologically active component, provided
4 that binding of the conjugate, fusion protein, or complex to pIgR reduces proteolytic
5 cleavage of secretory component (SC) by at least one-third compared to the cleavage of
6 SC from a cell in the absence of binding of the conjugate, fusion protein, or complex and
7 provided further that the conjugate, fusion protein, or complex does not substantially bind
8 to a stalk of said pIgR remaining after proteolytic cleavage under physiological
9 conditions.

1 44. A method of introducing a ligand into a cell expressing a polymeric
2 immunoglobulin receptor (pIgR) by attaching the ligand to a region of the pIgR, provided
3 that

4 (a) binding of the ligand reduces proteolytic cleavage of secretory
5 component (SC) by at least one-third compared to the cleavage of SC from a cell in the
6 absence of the ligand, and

7 (b) the ligand does not substantially bind to a stalk of said pIgR remaining
8 after proteolytic cleavage under physiological conditions,
9 thereby permitting introduction of the ligand into the cell.

1 45. A method of claim 44, wherein the ligand is an antibody.

1 46. A method of claim 45, wherein the ligand is a humanized antibody.

1 47. A method of claim 45, wherein the ligand is a scFv.

1 48. A method of claim 45, wherein the ligand is selected from the
2 group consisting of a recombinant single chain variable region fragment of an antibody
3 and a disulfide stabilized variable region.

1 49. A method of claim 44, wherein the ligand binds to a peptide
2 derived from human pIgR (SEQ ID NO:2), selected from the group consisting of:
3 Lys487-Arg603, Lys487-Glu607, Lys487-Val611, Lys487-Arg615, Lys487-Ala618,
4 Cys520-Arg603, Cys520-Glu607, Cys520-Val611, Cys520-Arg615, Cys520-Ala618,
5 Lys577-Arg603, Lys577-Glu607, Lys577-Val611, Lys577-Arg615, Lys577-Ala618,
6 Ser574-Arg603, Ser574-Glu607, Ser574-Val611, Ser574-Arg615, Ser574-Ala618,
7 Val560-Arg603, Val560-Glu607, Val560-Val611, Val560-Arg615, Val560-Ala618,
8 Cys544-Arg603, Cys544-Glu607, Cys544-Val611, Cys544-Arg615, and Cys544-Ala618.

1 50. The method of claim 44, wherein the ligand binds to an epitope of
2 pIgR selected from the group consisting of QDPRLF (SEQ ID NO:10), LDPRLF (SEQ
3 ID NO:11), KAIQDPRLF (SEQ ID NO:12), LDPRLFADERI (SEQ ID NO:13),
4 DENKANLDPRLF (SEQ ID NO:14), RLFADEREI (SEQ ID NO:15), and LDPRLFAD
5 (SEQ ID NO:16).

1 51. A method of claim 44, wherein the ligand is further defined as
2 having a binding component for selectively binding to a region of pIgR and a biologically
3 active component.

1 52. The method of claim 51, wherein the biologically active
2 component is selected from the group consisting of: a nucleic acid, a protein, a
3 radioisotope, a lipid, a carbohydrate, a peptidomimetic, an anti-inflammatory, an
4 antibiotic, and an anti-infective.

1 53. The method of claim 51, wherein the biologically active
2 component is a small molecule.

1 54. A method of claim 51, wherein the animal is a mammal.

1 55. A method of claim 51, wherein the biologically active component
2 is a nucleic acid encodes the wildtype cystic fibrosis transmembrane conductance
3 regulator.

- 1 56. A method of claim 44, wherein the cell is a mammalian cell.
- 1 57. A method of claim 56, wherein the cell is an epithelial cell.
- 1 58. The method of claim 44, wherein the ligand binds to the pIgR at
2 the apical surface of the cell.
- 1 59. The method of claim 59, wherein the ligand is transcytosed to the
2 basolateral side of the cell.
- 1 60. The method of claim 59, wherein the ligand is released from the
2 pIgR at the basolateral surface of the cell.
- 1 61. The method of claim 44, wherein the ligand is attached to the pIgR
2 at the basolateral surface of the cell.
- 1 62. The method of claim 44, wherein the SC exists in several forms in
2 an organ of interest, and provided that the ligand
3 (a) does not bind to the most abundant form of SC present in the organ of
4 interest, and
5 (b) does not bind to a stalk remaining on an extracellular surface of a cell
6 of the organ of interest after pIgR cleavage.
- 1 63. The method of claim 62, wherein the organ of interest is selected
2 from the group consisting of a small intestine, a large intestine, a liver-biliary tree, a
3 stomach, a salivary gland, a lung, a vagina, a uterus, a lacrimal gland, a mammary gland,
4 a nasal passage, and a sinus.
- 1 64. A method of attaching a ligand to a cell expressing a polymeric
2 immunoglobulin receptor comprising the step of binding the ligand to the receptor with
3 the provisos that
4 (a) the ligand reduces proteolytic cleavage of secretory component (SC) by
5 at least one-third compared to the cleavage of SC from a cell in the absence of the ligand,
6 and
7 (b) the ligand does not substantially bind to a stalk of said pIgR remaining

8 after proteolytic cleavage under physiological conditions,
9 thereby attaching the ligand to the cell.

1 65. The method of claim 64, wherein the ligand is internalized into the
2 cell after binding.

1 66. A method of attaching a conjugate, fusion protein, or complex to a
2 cell expressing a polymeric immunoglobulin receptor, said conjugate, fusion protein, or
3 complex comprising a ligand that binds to a region of pIgR and a biologically active
4 component, said method comprising the step of binding the ligand to the receptor with the
5 provisos that

6 (a) the ligand reduces proteolytic cleavage of secretory component (SC) by
7 at least one-third compared to the cleavage of SC from a cell in the absence of the ligand,
8 and

9 (b) the ligand does not substantially bind to a stalk of said pIgR remaining
10 after proteolytic cleavage under physiological conditions,
11 thereby attaching the conjugate, fusion protein, or complex to the cell.

1 67. A method of transcytosing a ligand from an apical to a basolateral
2 side of a cell of an organ of interest in an animal, which cell expresses a polymeric
3 immunoglobulin receptor (pIgR), by binding the ligand to a region of the polymeric
4 immunoglobulin receptor, with the provisos that

5 (a) the ligand does not substantially bind to a form of secretory component
6 which is the most abundant form present in the organ of interest under physiological
7 conditions and

8 (b) the ligand does not substantially bind to a stalk region of the pIgR,
9 thereby permitting introduction of the ligand into the cell.

1 68. A method of claim 67, wherein the ligand is an antibody.

1 69. A method of claim 68, wherein the ligand is a humanized antibody.

1 70. A method of claim 67, wherein the ligand is selected from the
2 group consisting of a recombinant single chain variable region fragment of an antibody
3 and a disulfide stabilized variable region fragment.

548
A20

71. A method of claim 67, wherein the ligand selectively binds to a peptide derived from human pIgR (SEQ ID NO:2), which peptide is selected from the group consisting of: Lys487-Arg603, Lys487-Glu607, Lys487-Val611, Lys487-Arg615, Lys487-Ala618, Cys520-Arg603, Cys520-Glu607, Cys520-Val611, Cys520-Arg615, Cys520-Ala618, Lys577-Arg603, Lys577-Glu607, Lys577-Val611, Lys577-Arg615, Lys577-Ala618, Ser574-Arg603, Ser574-Glu607, Ser574-Val611, Ser574-Arg615, Ser574-Ala618, Val560-Arg603, Val560-Glu607, Val560-Val611, Val560-Arg615, Val560-Ala618, Cys544-Arg603, Cys544-Glu607, Cys544-Val611, Cys544-Arg615, and Cys544-Ala618.

72. The method of claim 67, wherein the ligand binds to an epitope selected from the group consisting of QDPRLF (SEQ ID NO:10), LDPRLF (SEQ ID NO:11), KAIQDPRLF (SEQ ID NO:12), LDPRLFADERI (SEQ ID NO:13), DENKANLDPRLF (SEQ ID NO:14), RLFADEREI (SEQ ID NO:15), and LDPRLFAD (SEQ ID NO:16).

73. A method of claim 67 wherein the ligand is further defined as having a binding component for selectively binding to pIgR and a biologically active component.

74. A method of claim 73, wherein the biologically active component is selected from the group consisting of a nucleic acid, a peptide, a protein, a radioisotope, a lipid, a carbohydrate, a peptidomimetic, an anti-inflammatory, an antisense oligonucleotide, an antibiotic, and an anti-infective.

75. A method of claim 73, wherein the biologically active component is a small molecule

76. A method of claim 67, wherein the cell is a mammalian cell.

77. A method of claim 76, wherein the cell is an epithelial cell.

78. A method of claim 67, wherein the organ of interest is selected from the group consisting of a small intestine, a large intestine, a liver-biliary tree, a stomach, a salivary gland, a lung, a vagina, a uterus, a lacrimal gland, a mammary gland, a nasal passage, and a sinus.

1 79. A method of transcytosing a ligand from an apical to a basolateral
2 side of a cell of an organ of interest in an animal, which cell expresses a polymeric
3 immunoglobulin receptor (pIgR), which pIgR has an initial cleavage site which, upon
4 initial cleavage has a stalk region, the method comprising binding the ligand to a region
5 of the pIgR, with the provisos that

6 (a) the ligand does not substantially bind to a form of secretory component
7 which is the most abundant form present in the organ of interest under physiological
8 conditions;

9 (b) the ligand does not substantially bind to a stalk region of the pIgR; and

10 (c) the ligand does not bind to an extracellular epitope within the first 31
11 amino acids that are cell membrane proximal to the initial cleavage site of the pIgR,
12 thereby permitting introduction of the ligand into the cell.

1 80. A method of transcytosing a ligand from an apical to a basolateral
2 side of a cell of an organ of interest in an animal, which cell expresses a polymeric
3 immunoglobulin receptor (pIgR), by attaching the ligand to a region of the pIgR, provided
4 that

5 (a) binding of the ligand reduces proteolytic cleavage of secretory
6 component (SC) by at least one-third compared to the cleavage of SC from a cell in the
7 absence of the ligand, and

8 (b) the ligand does not substantially bind to a stalk of said pIgR remaining
9 after proteolytic cleavage under physiological conditions,
10 thereby permitting transcytosis of the ligand from the apical side to the basolateral side of
11 the cell.

1 81. A method of claim 80, wherein the ligand is an antibody.

1 82. A method of claim 81, wherein the ligand is a humanized antibody.

1 83. A method of claim 81, wherein the ligand is a scFv.

1 84. A method of claim 80, wherein the ligand is selected from the
2 group consisting of a recombinant single chain variable region fragment of an antibody
3 and a disulfide stabilized variable region.

1 85. A method of claim 80, wherein the ligand binds to a peptide
2 derived from human pIgR (SEQ ID NO:2), selected from the group consisting of:
3 Lys487-Arg603, Lys487-Glu607, Lys487-Val611, Lys487-Arg615, Lys487-Ala618,
4 Cys520-Arg603, Cys520-Glu607, Cys520-Val611, Cys520-Arg615, Cys520-Ala618,
5 Lys577-Arg603, Lys577-Glu607, Lys577-Val611, Lys577-Arg615, Lys577-Ala618,
6 Ser574-Arg603, Ser574-Glu607, Ser574-Val611, Ser574-Arg615, Ser574-Ala618,
7 Val560-Arg603, Val560-Glu607, Val560-Val611, Val560-Arg615, Val560-Ala618,
8 Cys544-Arg603, Cys544-Glu607, Cys544-Val611, Cys544-Arg615, and Cys544-Ala618.

1 86. The method of claim 80, wherein the ligand binds to an epitope of
2 pIgR selected from the group consisting of QDPRLF (SEQ ID NO:10), LDPRLF (SEQ
3 ID NO:11), KAIQDPRLF (SEQ ID NO:12), LDPRLFADERI (SEQ ID NO:13),
4 DENKANLDPRLF (SEQ ID NO:14), RLFADEREI (SEQ ID NO:15), and LDPRLFAD
5 (SEQ ID NO:16).

1 87. A method of claim 80, wherein the ligand is further defined as
2 having a binding component for selectively binding to a region of pIgR and a biologically
3 active component.

1 88. A method of claim 87, wherein said biologically active component
2 is selected from the group consisting of a nucleic acid, a peptide, a protein, a
3 radioisotope, a lipid, a carbohydrate, a peptidomimetic, an anti-inflammatory, an
4 antisense oligonucleotide, an antibiotic, and an anti-infective.

1 89. A method of claim 87, wherein said biologically active component
2 is a small molecule.

1 90. A method of claim 80, wherein the animal is a mammal.

1 91. A method of claim 90, wherein the cell is a mammalian cell.

1 92. A method of claim 91, wherein the cell is an epithelial cell.

1 93. A method of increasing the rate by which a first ligand which binds
2 to secretory component (SC) is transcytosed from an apical to a basolateral side of a cell
3 of an organ of interest in an animal, which cell expresses a polymeric immunoglobulin

4 receptor (pIgR) from an apical surface by
5 (a) binding the pIgR at the apical side of said cell with a second ligand,
6 which second ligand inhibits proteolytic cleavage of SC by at least one-third, and further
7 which second ligand does not substantially bind to a stalk remaining attached to the cell
8 after proteolytic cleavage, and
9 (b) binding the first ligand to the SC,
10 thereby permitting transcytosis of the SC to which the first ligand has bound from the
11 apical to the basolateral side of said cell.

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000